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| NEWS | 4 | Feb 16 | TOXLINE no longer being updated |
| NEWS | 5 | Apr 23 | Search Derwent WPINDEX by chemical structure |
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| NEWS | 7 | May 07 | DGENE Reload |
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=> s fluorescence resonance energy transfer and melting temperature

L1 45 FLUORESCENCE RESONANCE ENERGY TRANSFER AND MELTING TEMPERATURE

=> s l1 and (multiplex or multiple loci)

L2 11 L1 AND (MULTIPLEX OR MULTIPLE LOCI)

=> dup rem l2

PROCESSING COMPLETED FOR L2
L3 10 DUP REM L2 (1 DUPLICATE REMOVED)

=> d 13 1-10 bib ab

L3 ANSWER 1 OF 10 USPATFULL
AN 2001:86217 USPATFULL
TI Fluorescent donor-acceptor pair with low spectral overlap
IN Wittwer, Carl T., Salt Lake City, UT, United States
PA University of Utah Research Foundation, Salt Lake City, UT, United States (U.S. corporation)
PI US 6245514 B1 20010612
AI US 1999-398629 19990917 (9)
RLI Division of Ser. No. US 1997-869276, filed on 4 Jun 1997
Continuation-in-part of Ser. No. US 1997-818267, filed on 17 Mar 1997
Continuation-in-part of Ser. No. US 1996-658993, filed on 4 Jun 1996, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Horlick, Kenneth R.
LREP Barnes & Thornburg
CLMN Number of Claims: 24
ECL Exemplary Claim: 2
DRWN 71 Drawing Figure(s); 52 Drawing Page(s)
LN.CNT 3355
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to **fluorescence resonance energy transfer** pairs for detecting the presence of a target analyte wherein the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption spectrum overlap by less than 25%. In a preferred embodiment, the present invention relates to the use of fluorescein and Cy5 or Cy5.5 as **fluorescence resonance energy transfer** pairs for use as labels on oligonucleotides for analysis of a nucleic acid locus during amplification.

L3 ANSWER 2 OF 10 USPATFULL
AN 2001:75133 USPATFULL
TI Detection of nucleic acid hybrids
IN Shultz, John William, Verona, WI, United States
Lewis, Martin K., Madison, WI, United States
Leippe, Donna, Madison, WI, United States
Mandrekar, Michelle, Oregon, WI, United States
Kephart, Daniel, Cottage Grove, WI, United States
Rhodes, Richard Byron, Madison, WI, United States
Andrews, Christine Ann, Cottage Grove, WI, United States

Hartnett, James Robert, Madison, WI, United States
Gu, Trent, Madi[redacted], WI, United States
Olson, Ryan J., [redacted]adison, WI, United States
Wood, Keith V., Madison, WI, United States
Welch, Roy, Palo Alto, CA, United States
PA Promega Corporation, Madison, WI, United States (U.S. corporation)
PI US 6235480 B1 20010522
AI US 1999-358972 19990721 (9)
RLI Continuation-in-part of Ser. No. US 1999-252436, filed on 18 Feb 1999
Continuation-in-part of Ser. No. US 1998-42287, filed on 13 Mar 1998
DT Utility
FS Granted
EXNAM Primary Examiner: Fredman, Jeffrey; Assistant Examiner: Chakrabarti, Arun kr.
LREP Welsh & Katz, Ltd.
CLMN Number of Claims: 170
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 12088
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Processes are disclosed using the depolymerization of a nucleic acid hybrid to qualitatively and quantitatively analyze for the presence of a predetermined nucleic acid. Applications of those processes include the detection of single nucleotide polymorphisms, identification of single base changes, speciation, determination of viral load, genotyping, medical marker diagnostics, and the like.

L3 ANSWER 3 OF 10 USPATFULL
AN 2001:71314 USPATFULL
TI PCR method for nucleic acid quantification utilizing second or third order rate constants
IN Wittwer, Carl T., Salt Lake City, UT, United States
Ririe, Kirk M., Idaho Falls, ID, United States
Rasmussen, Randy P., Salt Lake City, UT, United States
PA University of Utah Research Foundation, Salt Lake City, UT, United States (U.S. corporation)
PI US 6232079 B1 20010515
AI US 2000-635344 20000809 (9)
RLI Division of Ser. No. US 1997-869276, filed on 4 Jun 1997
Continuation-in-part of Ser. No. US 1997-818267, filed on 17 Mar 1997
Continuation-in-part of Ser. No. US 1996-658993, filed on 4 Jun 1996, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Horlick, Kenneth R.
LREP Barnes & Thornburg
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 71 Drawing Figure(s); 52 Drawing Page(s)
LN.CNT 3328
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a method of determining the concentration of a nucleic acid product that had been amplified through polymerase chain reaction (PCR). More particularly, the present invention relates to a method wherein a rate constant is determined for a known concentration of amplified product by monitoring the rate of hybridization of the known concentration, and then the concentration of an unknown concentration of a nucleic acid product can be determined by determining the rate of annealing for the unknown concentration, and calculating the concentration from the rate of annealing and the rate constant.

L3 ANSWER 4 OF 10 USPATFULL
AN 2001:43982 USPATFULL

TI Self-primed amplification system
IN Chou, Quin, Dal[redacted]City, CA, United States
Maa, Joe, San [redacted]ho, CA, United States
Chang, Charlie, Saratoga, CA, United States
PA Maxim Biotech, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 6207424 B1 20010327
AI US 1999-447942 19991123 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Taylor, Janell E.
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 52
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 1435
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods and compositions for copying a target nucleic acid
using a self-priming primer. Single and different target nucleic acids can be copied using the disclosed methods and compositions.

L3 ANSWER 5 OF 10 USPATFULL
AN 2001:7838 USPATFULL
TI Monitoring amplification of DNA during PCR
IN Wittwer, Carl T., Salt Lake City, UT, United States
Ririe, Kirk M., Idaho Falls, ID, United States
Rasmussen, Randy P., Salt Lake City, UT, United States
PA University of Utah Research Foundation, Salt Lake City, UT, United States (U.S. corporation)
PI US 6174670 B1 20010116
AI US 1997-869276 19970604 (8)
RLI Continuation-in-part of Ser. No. US 1997-818267, filed on 17 Mar 1997
Continuation-in-part of Ser. No. US 1996-658993, filed on 4 Jun 1996, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Horlick, Kenneth R.
LREP Barnes & Thornburg
CLMN Number of Claims: 107
ECL Exemplary Claim: 1
DRWN 67 Drawing Figure(s); 52 Drawing Page(s)
LN.CNT 4094
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of monitoring hybridization during polymerase chain reaction are
disclosed. These methods are achieved with rapid thermal cycling and use
of double stranded DNA dyes or specific hybridization probes. A **fluorescence resonance energy transfer** pair comprises fluorescein and Cy5 or Cy5.5. Methods for quantitating amplified DNA and determining its purity are carried out by analysis of melting and reannealing curves.

L3 ANSWER 6 OF 10 USPATFULL
AN 2000:146100 USPATFULL
TI **Multiplex** genotyping using fluorescent hybridization probes
IN Wittwer, Carl T., Salt Lake City, UT, United States
Bernard, Philip S., Salt Lake City, UT, United States
PA University of Utah Research Foundation, Salt Lake City, UT, United States (U.S. corporation)
PI US 6140054 20001031
AI US 1998-164023 19980930 (9)
DT Utility
FS Granted

EXNAM Primary Examiner: Horlick, Kenneth R.; Assistant Examiner: Taylor, Janell E.
LREP Barnes & Thornburg
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 26 Drawing Figure(s); 23 Drawing Page(s)
LN.CNT 1890
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a mutation detection kit and method of analyzing **multiple loci** of one or more nucleic acid sequences for the presence of mutations or polymorphisms. More particularly, the present invention relates to the use of the polymerase chain reaction (PCR) and fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of the hybridization probes.

L3 ANSWER 7 OF 10 USPATFULL
AN 2000:131593 USPATFULL
TI Oligonucleotides containing pyrazolo[3,4-D]pyrimidines for hybridization and mismatch discrimination
IN Meyer, Jr., Rich B., Bothell, WA, United States
Afonina, Irina A., Mill Creek, WA, United States
Kutyavin, Igor V., Bothell, WA, United States
PA Epoch Pharmaceuticals, Inc., Redmond, WA, United States (U.S. corporation)
PI US 6127121 20001003
AI US 1998-54830 19980403 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Marschel, Ardin H.
LREP Morrison & Foerster LLP
CLMN Number of Claims: 46
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1457
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Oligonucleotides in which one or more purine residues are substituted by pyrazolo[3,4-d]pyrimidines exhibit improved hybridization properties. Oligonucleotides containing pyrazolo[3,4-d]pyrimidine base analogues have higher melting temperatures than unsubstituted oligonucleotides of identical sequence. Thus, in assays involving hybridization of an oligonucleotide probe to a target polynucleotide sequence, higher signals are obtained. In addition, mismatch discrimination is enhanced when pyrazolo[3,4-d]pyrimidine-containing oligonucleotides are used as hybridization probes, making them useful as probes and primers for hybridization, amplification and sequencing procedures, particularly those in which single- or multiple-nucleotide mismatch discrimination is required.

L3 ANSWER 8 OF 10 USPATFULL
AN 2000:105661 USPATFULL
TI Detectably labeled, dual conformation oligonucleotide probes, assays and kits
IN Tyagi, Sanjay, New York, NY, United States
Kramer, Fred R., Riverdale, NY, United States
Lizardi, Paul M., Cuernavaca, Mexico
PA The Public Health Research Institute of the City of New York, Inc., NY, United States (U.S. corporation)
PI US 6103476 20000815

AI US 1999-268402 19990315 (9)
RLI Continuation of Ser. No. US 1995-439819, filed on 12 May 1995, now patented, Pat. No. US 5925517 which is a continuation-in-part of Ser. No. US 1993-152006, filed on 12 Nov 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Campbell, Eggerton A.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 14 Drawing Page(s)
LN.CNT 2522
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Unimolecular and bimolecular hybridization probes for the detection of nucleic acid target sequences comprise a target complement sequence, an affinity pair holding the probe in a closed conformation in the absence of target sequence, and either a label pair that interacts when the probe is in the closed conformation or, for certain unimolecular probes, a non-interactive label. Hybridization of the target and target complement sequences shifts the probe to an open conformation. The shift is detectable due to reduced interaction of the label pair or by detecting a signal from a non-interactive label. Certain unimolecular probes can discriminate between target and non-target sequences differing by as little as one nucleotide. Also, universal stems and kits useful for constructing said probes. Also, assays utilizing said probes and kits for performing such assays.

L3 ANSWER 9 OF 10 USPATFULL
AN 1999:99757 USPATFULL
TI Ligation assembly and detection of polynucleotides on solid-support
IN Hunkapiller, Michael W., San Carlos, CA, United States
Hiatt, Andrew C., San Diego, CA, United States
PA The Porkin-Elmer Corporation, Foster City, CA, United States (U.S. corporation)
PI US 5942609 19990824
AI US 1998-191390 19981112 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Marschel, Ardin H.
LREP Grossman, Paul D.
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 32 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 1069
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention concerns methods of assembly of a polynucleotide on a solid-support by performing steps of annealing, ligation, and extension. The steps may be repeated in a cyclical manner to assemble immobilized double- or single-stranded polynucleotides with functional gene properties. The immobilized polynucleotides may be amplified by the polymerase chain reaction, and detected and quantitated by an exonuclease assay with a self-quenching, fluorescent probe. The polynucleotide may be cleaved from the solid-support by chemical or enzymatic cleavage.

L3 ANSWER 10 OF 10 MEDLINE DUPLICATE 1
AN 1998449367 MEDLINE
DN 98449367 PubMed ID: 9777937
TI Homogeneous **multiplex** genotyping of hemochromatosis mutations with fluorescent hybridization probes.
AU Bernard P S; Ajioka R S; Kushner J P; Wittwer C T

CS Department of Pathology, University of Utah Medical School, Salt Lake City
84132, USA.
NC DK20630 (NIDDK)
GM51647 (NIGMS)
RR00064 (NCRR)
+
SO AMERICAN JOURNAL OF PATHOLOGY, (1998 Oct) 153 (4) 1055-61.
Journal code: 3RS; 0370502. ISSN: 0002-9440.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199810
ED Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981028
AB **Multiplex** polymerase chain reaction amplification and genotyping by fluorescent probe **melting temperature** (Tm) was used to simultaneously detect multiple variants in the hereditary hemochromatosis gene. Homogenous real-time analysis by fluorescent melting curves has previously been used to genotype single base mismatches; however, the current method introduces a new probe design for **fluorescence resonance energy transfer** and demonstrates allele multiplexing by Tm for the first time. The new probe design uses a 3'-fluorescein-labeled probe and a 5'-Cy5-labeled probe that are in fluorescence energy transfer when hybridized to the same strand internal to an unlabeled primer set. Two hundred and fifty samples were genotyped for the C282Y and H63D hemochromatosis causing mutations by fluorescent melting curves. Multiplexing was performed by including two primer sets and two probe sets in a single tube. In clinically defined groups of 117 patients and 56 controls, the C282Y mutation was found in 87% (204/234) of patient chromosomes, and the relative penetrance of the H63D mutation was 2.4% of the homozygous C282Y mutation. Results were confirmed by restriction enzyme digestion and agarose gel electrophoresis.
In addition, the probe covering the H63D mutation unexpectedly identified the A193T polymorphism in some samples. This method is amenable to multiplexing and has promise for scanning unknown mutations.

=> d l3 1 kwic

L3 ANSWER 1 OF 10 USPATFULL
AB The present invention relates to **fluorescence resonance energy transfer** pairs for detecting the presence of a target analyte wherein the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption. . . . than 25%. In a preferred embodiment, the present invention relates to the use of fluorescein and Cy5 or Cy5.5 as **fluorescence resonance energy transfer** pairs for use as labels on oligonucleotides for analysis of a nucleic acid locus during amplification.
SUMM . . . GC/AT ratio, length, and sequence, and can be used to differentiate amplification products separated by less than 2.degree. C. in **melting temperature**. Desired products can be distinguished from undesired products, including primer dimers. Analysis of melting curves can be used to extend the dynamic range of quantitative PCR and to differentiate different products in

**multiplex** amplification. Using double strand dyes, product denaturation, [redacted]nnealing and extension can be [redacted]llowed within each cycle. Continu[redacted] monitoring of fluorescence. . . .

SUMM . . . hybridizing to the amplified products such that the probe melts from the amplified product of the selected template at a **melting temperature** that is distinguishable from the **melting temperature** at which the probe melts from the amplified product of the competitive template,

SUMM A **fluorescence resonance energy transfer** pair is disclosed wherein the pair comprises a donor fluorophore having an emission spectrum and an acceptor fluorophore having an. . . M.sup.-1 cm.sup.-1, wherein the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption spectrum overlap less than 25%. One illustrative **fluorescence resonance energy transfer** pair described is where the donor fluorophore is fluorescein and the acceptor fluorophore is Cy5 or Cy5.5.

DETD As used herein, "**fluorescence resonance energy transfer** relationship" and similar terms refer to adjacent hybridization of an oligonucleotide labeled with a donor fluorophore and another oligonucleotide labeled. . .

DETD . . . depend primarily on GC content and length. If a probe is designed to hybridize internally to the PCR product, the **melting temperature** of the probe also depends on GC content, length, and degree of complementarity to the target. Fluorescence probes compatible with. . .

DETD **Fluorescence resonance energy transfer** can occur between 2 fluorophores if they are in physical proximity and the emission spectrum of one fluorophore overlaps the excitation spectrum of the other. Introductory theory on **fluorescence resonance energy transfer** can be found in many recent review articles. The rate of resonance energy transfer is:

DETD where K is a proportionality constant. De-excitation of the donor will occur by **fluorescence, resonance energy transfer**, and other processes summarized as thermal quenching. If p.sub.F =probability of resonance energy transfer, and p.sub.TD =probability of donor thermal. . .

DETD . . . low spectral overlap. In a later example, the utility of fluorescein and Cy5.5 as labels on hybridization probes is demonstrated.

**Fluorescence resonance energy transfer** can be used to monitor nucleic acid hybridization even when the interacting dyes have low spectral overlap. The use of. . .

DETD In additional experiments, the number of bases separating the Cy5-label and the fluorescein label were varied. The best **fluorescence resonance energy transfer** was observed with about 4-6 bases between the fluorophores, although a signal was detectable up to at least 15 bases.

DETD . . . melt varies over a large range. Using empirical formulas known in the art, the effect of GC content on the **melting temperature** (Tm) of DNA predicts that a 0% GC duplex would melt 41.degree. C. lower than a 100% GC duplex. Given. . .

DETD . . . single reaction tube. Melting curves obtained by continuous monitoring of PCR reactions according to the present invention are useful in **multiplex** PCR.

DETD **Multiplex** amplification is useful in cases where an internal control is needed during amplification. For example, many translocations are detectable by. . .

DETD Relative quantification of two PCR products is important in many quantitative PCR applications. **Multiplex** amplification of two

or more products followed by integration of the areas under the melting peaks will be extremely useful. . .

DETD . . . a hybridization probe is placed at a mutation site, single base mutations are detectable as a shift in the probe **melting temperature**.

DETD . . . conceivably contain as few as about 10 nucleotide residues, however, possible disadvantages of such short oligonucleotides include low specificity, low **melting temperature**, and increased background. Oligonucleotides larger than 40 residues could also be used, but would be unnecessarily expensive. Thus, the limits.

.

DETD . . . Further, the same technique can be used to detect insertions and deletions by designing the hybridization probe so that it **melting temperature** changes when the mutation or polymorphism is present. The invention can be used to detect any known mutation where a probe can be designed to differ in **melting temperature** when hybridized to mutant vs wild type.

DETD The discriminatory power of hybridization probes is also used to great advantage in **multiplex** or competitive PCR. For example, an artificial template is designed with a single internal base change and a hybridization probe. . .

DETD . . . within the scope of the present invention is to use probe annealing rates to determine product concentrations. The rate of **fluorescence resonance energy transfer** is followed over time after a quick drop to a probe annealing temperature that is greater than the primer annealing. . .

DETD The resulting temperature/time plot (FIG. 52) shows a characteristic increase in the **melting temperature** after cycle 20 as the concentration of amplification product increases. Product Tm is a function of product concentration.

DETD . . . available in the art. For example, the present invention provides single-color fluorescence methods to monitor product purity, relative quantitation by **multiplex** PCR or competitive PCR, absolute product quantification by reannealing kinetics, and an improved method for initial template quantification by fluorescence vs cycle number plots. The present invention also provides dual-color, sequence-specific methods for sequence variation detection, relative quantitation by **multiplex** PCR or competitive PCR, product quantification by probe annealing kinetics, and initial template quantification by fluorescence vs cycle number plots.

DETD . . . with respect to temperature is used to identify products by melting curves. In addition, relative product quantification is achieved by **multiplex** amplification of two or more different products that differ in Tm. Further, competitive PCR is performed by altering the sequence. . .

DETD . . . probes, and (2) one labeled probe that hybridizes to a single stranded PCR product that incorporates a labeled primer. The **melting temperature** of sequence-specific probes identifies and discriminates products during PCR. DNA polymorphisms or mutations, including single base mutations, are detected by probe Tm shifts. In addition, relative product quantification is achieved by **multiplex** amplification of at least two different products with one or more probes that melt from their respective products at different. . .

DETD When **multiplex** analysis in one PCR reaction is desired, it is common practice to use different fluorescent labels with distinguishable

emission spectra. . .
CLM What is claime[redacted]s:
. . . said first [redacted]be and said second probe are p[redacted]tioned so that the fluorescein and Cy5 or Cy5.5 are in a **fluorescence resonance energy transfer** relationship.

2. A **fluorescence resonance energy transfer** pair for producing a fluorescent signal under predetermined conditions, said pair comprising a donor fluorophore having an emission spectrum and. . . acceptor fluorophore's absorption spectrum overlap less than 25%, and wherein under the predetermined conditions the donor fluorophore is in a **fluorescence resonance energy transfer** relationship with the acceptor fluorophore.

3. The **fluorescence resonance energy transfer** pair of claim 2 wherein the donor fluorophore is fluorescein and the acceptor fluorophore is Cy5 or Cy5.5.

4. The **fluorescence resonance energy transfer** pair of claim 2 wherein the donor fluorophore is provided as a label on a first oligonucleotide, the acceptor fluorophore. . .

5. The **fluorescence resonance energy transfer** pair of claim 2 wherein the donor fluorophore and acceptor fluorophore are provided on a single oligonucleotide probe and held. . .

6. The **fluorescence resonance energy transfer** pair of claim 2 wherein the acceptor fluorophore has an extinction coefficient greater than 100,000 M.sup.-1 cm.sup.-1.

7. A **fluorescence resonance energy transfer** pair for producing a fluorescent signal under predetermined conditions, said pair comprising a donor fluorophore having an emission spectrum and. . . acceptor fluorophore's absorption spectrum overlap by about 15%, and wherein under the predetermined conditions the donor fluorophore is in a **fluorescence resonance energy transfer** relationship with the acceptor fluorophore.

8. The **fluorescence resonance energy transfer** pair of claim 7 wherein the donor fluorophore is fluorescein and the acceptor fluorophore is Cy5.5.

. . . emission spectrum; and an acceptor fluorophore having an absorption spectrum, wherein the donor fluorophore and the acceptor fluorophore comprise a **fluorescence resonance energy transfer** pair such that the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption spectrum overlap by less than 25%, and. . .

. . . emission spectrum and an acceptor fluorophore having an absorption spectrum wherein the donor fluorophore and the acceptor fluorophore comprise a **fluorescence resonance energy transfer** pair such that the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption spectrum overlap by less than 25%, and. . .

=> d l3 kwic

L3 ANSWER 1 OF 10 USPATFULL
AB The present invention relates to **fluorescence resonance energy transfer** pairs for detecting the presence of a target analyte wherein the donor

fluorophore's emission spectrum and the acceptor fluorophore's absorption. . . . than 25%. In a preferred emb[redacted]ment, the present invention rela[redacted]s to the use of fluorescein and [redacted]y5 or Cy5.5 as **fluorescence resonance energy transfer** pairs for use as labels on oligonucleotides for analysis of a nucleic acid locus during amplification.

SUMM . . . GC/AT ratio, length, and sequence, and can be used to differentiate amplification products separated by less than 2.degree. C. in **melting temperature**. Desired products can be distinguished from undesired products, including primer dimers. Analysis of melting curves can be used to extend the dynamic range of quantitative PCR and to differentiate different products in **multiplex** amplification. Using double strand dyes, product denaturation, reannealing and extension can be followed within each cycle. Continuous monitoring of fluorescence. . .

SUMM . . . hybridizing to the amplified products such that the probe melts from the amplified product of the selected template at a **melting temperature** that is distinguishable from the **melting temperature** at which the probe melts from the amplified product of the competitive template,

SUMM A **fluorescence resonance energy transfer** pair is disclosed wherein the pair comprises a donor fluorophore having an emission spectrum and an acceptor fluorophore having an. . . M.sup.-1 cm.sup.-1, wherein the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption spectrum overlap less than 25%. One illustrative **fluorescence resonance energy transfer** pair described is where the donor fluorophore is fluorescein and the acceptor fluorophore is Cy5 or Cy5.5.

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DETD . . . depend primarily on GC content and length. If a probe is designed to hybridize internally to the PCR product, the **melting temperature** of the probe also depends on GC content, length, and degree of complementarity to the target. Fluorescence probes compatible with. . .

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DETD where K is a proportionality constant. De-excitation of the donor will occur by **fluorescence, resonance energy transfer**, and other processes summarized as thermal quenching. If p.sub.F =probability of resonance energy transfer, and p.sub.TD =probability of donor thermal. . .

DETD . . . low spectral overlap. In a later example, the utility of fluorescein and Cy5.5 as labels on hybridization probes is demonstrated. **Fluorescence resonance energy transfer** can be used to monitor nucleic acid hybridization even when the interacting dyes have low spectral overlap. The use of. . .

DETD In additional experiments, the number of bases separating the Cy5-label and the fluorescein label were varied. The best **fluorescence resonance energy transfer** was observed with about 4-6 bases between the fluorophores, although a signal was

detectable up to at least 15 bases.

DETD . . . melt [redacted]ies over a large range. Using [redacted]irical formulas known in the art, th[redacted]effect of GC content on the **mel[redacted]ng temperature** (Tm) of DNA predicts that a 0% GC duplex would melt 41.degree. C. lower than a 100% GC duplex. Given. . .

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DETD Relative quantification of two PCR products is important in many quantitative PCR applications. **Multiplex** amplification of two or more products followed by integration of the areas under the melting peaks will be extremely useful. . .

DETD . . . a hybridization probe is placed at a mutation site, single base mutations are detectable as a shift in the probe **melting temperature**.

DETD . . . conceivably contain as few as about 10 nucleotide residues, however, possible disadvantages of such short oligonucleotides include low specificity, low **melting temperature**, and increased background. Oligonucleotides larger than 40 residues could also be used, but would be unnecessarily expensive. Thus, the limits.

.

DETD . . . Further, the same technique can be used to detect insertions and deletions by designing the hybridization probe so that it **melting temperature** changes when the mutation or polymorphism is present. The invention can be used to detect any known mutation where a probe can be designed to differ in **melting temperature** when hybridized to mutant vs wild type.

DETD The discriminatory power of hybridization probes is also used to great advantage in **multiplex** or competitive PCR. For example, an artificial template is designed with a single internal base change and a hybridization probe. . .

DETD . . . within the scope of the present invention is to use probe annealing rates to determine product concentrations. The rate of **fluorescence resonance energy transfer** is followed over time after a quick drop to a probe annealing temperature that is greater than the primer annealing. . .

DETD The resulting temperature/time plot (FIG. 52) shows a characteristic increase in the **melting temperature** after cycle 20 as the concentration of amplification product increases. Product Tm is a function of product concentration.

DETD . . . available in the art. For example, the present invention provides single-color fluorescence methods to monitor product purity, relative quantitation by **multiplex** PCR or competitive PCR, absolute product quantification by reannealing kinetics, and an improved method for initial template quantification by fluorescence vs cycle number plots. The present invention also provides dual-color, sequence-specific methods for sequence variation detection, relative quantitation by **multiplex** PCR or competitive PCR, product quantification by probe annealing kinetics, and initial template quantification by fluorescence vs cycle number plots.

DETD . . . with respect to temperature is used to identify products by melting curves. In addition, relative product quantification is achieved by **multiplex** amplification of two or more different products that differ in Tm. Further, competitive PCR is performed by altering the

sequence. . .

DETD . . . probe and (2) one labeled probe that hybridizes to a single stranded PCR product that incorporates a labeled primer. The **melting temperature** of sequence-specific probes identifies and discriminates products during PCR. DNA polymorphisms or mutations, including single base mutations, are detected by probe Tm shifts. In addition, relative product quantification is achieved by **multiplex** amplification of at least two different products with one or more probes that melt from their respective products at different. . .

DETD When **multiplex** analysis in one PCR reaction is desired, it is common practice to use different fluorescent labels with distinguishable emission spectra. . .

CLM What is claimed is:

. . . said first probe and said second probe are positioned so that the fluorescein and Cy5 or Cy5.5 are in a **fluorescence resonance energy transfer** relationship.

2. A **fluorescence resonance energy transfer** pair for producing a fluorescent signal under predetermined conditions, said pair comprising a donor fluorophore having an emission spectrum and. . . acceptor fluorophore's absorption spectrum overlap less than 25%, and wherein under the predetermined conditions the donor fluorophore is in a **fluorescence resonance energy transfer** relationship with the acceptor fluorophore.

3. The **fluorescence resonance energy transfer** pair of claim 2 wherein the donor fluorophore is fluorescein and the acceptor fluorophore is Cy5 or Cy5.5.

4. The **fluorescence resonance energy transfer** pair of claim 2 wherein the donor fluorophore is provided as a label on a first oligonucleotide, the acceptor fluorophore. . .

5. The **fluorescence resonance energy transfer** pair of claim 2 wherein the donor fluorophore and acceptor fluorophore are provided on a single oligonucleotide probe and held. . .

6. The **fluorescence resonance energy transfer** pair of claim 2 wherein the acceptor fluorophore has an extinction coefficient greater than 100,000 M.sup.-1 cm.sup.-1.

7. A **fluorescence resonance energy transfer** pair for producing a fluorescent signal under predetermined conditions, said pair comprising a donor fluorophore having an emission spectrum and. . . acceptor fluorophore's absorption spectrum overlap by about 15%, and wherein under the predetermined conditions the donor fluorophore is in a **fluorescence resonance energy transfer** relationship with the acceptor fluorophore.

8. The **fluorescence resonance energy transfer** pair of claim 7 wherein the donor fluorophore is fluorescein and the acceptor fluorophore is Cy5.5.

. . . emission spectrum; and an acceptor fluorophore having an absorption spectrum, wherein the donor fluorophore and the acceptor fluorophore comprise a **fluorescence resonance energy transfer** pair such that the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption spectrum overlap by less than 25%, and. . .

. . . emission spectrum and an acceptor fluorophore having an absorption spectrum wherein the donor fluorophore and the acceptor fluorophore

comprise a **fluorescence resonance energy**
**transfer** pair su[redacted] that the donor fluorophore's e[redacted]sion
spectrum and t[redacted] acceptor fluorophore's absorpt[redacted]n spectrum overlap by
less than 25%, and. . .

=> d l3 6 kwic

L3 ANSWER 6 OF 10 USPATFULL
TI **Multiplex** genotyping using fluorescent hybridization probes
AB The present invention is directed to a mutation detection kit and
method
of analyzing **multiple loci** of one or more nucleic
acid sequences for the presence of mutations or polymorphisms. More
particularly, the present invention relates. . .
SUMM The present invention is directed to a method of analyzing
**multiple loci** of one or more nucleic acid sequences
for the presence of mutations or polymorphisms. More particularly, the
present invention relates. . .
SUMM . . . PCR-restriction fragment length analysis. All these methods
require time consuming multiple manual steps. One alternative method of
genotyping uses the **melting temperature** of
fluorescent hybridization probes that hybridize to a PCR amplified
targeted region of genome/nucleic acid sequence to identify mutations
and. . .
SUMM . . . raising the temperature of the DNA containing sample to a
denaturing temperature where the two DNA strands separate (i.e. the "
**melting temperature** of the DNA") and then the sample
is cooled to a lower temperature that allows the specific primers to
attach. . .
SUMM **Fluorescence resonance energy**
**transfer** (FRET) occurs between two fluorophores when they are in
physical proximity to one another and the emission spectrum of one. .
.
SUMM **Fluorescence resonance energy**
**transfer** can be used as a labeling system for detecting specific
sequences of DNA. In combination with standard melting curve analysis,.
. .
DRWD FIG. 16 is a schematic representation showing primer and probe
placement
for **multiplex** amplification and genotyping of HFE. Upstream
(U) and downstream (D) primers are illustrated with respect to exon
boundaries. Regions of. . . were amplified for analysis of the H63D
(C187G) and C282Y (G845A) mutations, respectively. The fluorescein (F)
labeled probes are in **fluorescence resonance**
**energy transfer** with the more thermally stable Cy5 (Y)
labeled probes. The fluorescein labeled probes form a single mismatch
when hybridizing to. . .
DRWD FIG. 20 shows homogenous **multiplex** genotyping by derivative
melting curves for 4 alleles. Shown are 4 samples with different
C282Y/H63D genotypes: homozygous C187 (-- --. . .
DETD As used herein, "**fluorescence resonance**
**energy transfer** pair" refers to a pair of fluorophores
comprising a donor fluorophore and acceptor fluorophore, wherein the
donor fluorophore is capable. . . In other words the emission
spectrum of the donor fluorophore overlaps the absorption spectrum of
the acceptor fluorophore. In preferred **fluorescence**
**resonance energy transfer** pairs, the
absorption spectrum of the donor fluorophore does not substantially
overlap the absorption spectrum of the acceptor fluorophore.
DETD As used herein, "**fluorescence resonance**
**energy transfer** relationship" and similar terms refer
to a donor fluorophore and acceptor fluorophore positioned in
sufficient

proximity and proper orientation to. . .

DETD . . . used herein, "FRET oligonucleotide pair" refers to the donor oligonucleotide probe and the acceptor oligonucleotide probe pair that form a **fluorescence resonance energy transfer** relationship when the donor oligonucleotide probe and the acceptor oligonucleotide probe are both hybridized to their complementary target nucleic acid. . .

DETD As used herein, "**melting temperature** of the FRET oligonucleotide pair" and "**melting temperature** of the set of donor oligonucleotide probe and acceptor oligonucleotide probe", defines the lowest temperature that will disrupt the hybridization. . .

DETD The present invention is directed to reagents and a method for screening **multiple loci** of nucleic acid sequences for the presence of mutations or polymorphisms. More particularly, the present invention allows for a rapid procedure, that can be entirely conducted within a single reaction vessel, for detecting mutations and polymorphisms at **multiple loci** of a genomic DNA sample prepared from an individual organism. The method comprises the steps of combining a biological sample. . .

DETD **Fluorescence resonance energy transfer** can be used to monitor nucleic acid hybridization even when the interacting dyes have low spectral overlap. The use of. . .

DETD . . . be shorter in length, down to about 10 nucleotide residues. Possible disadvantages of such shorter oligonucleotides include low specificity, low **melting temperature**, and increased background. Oligonucleotides larger than 40 residues could also be used, but would be unnecessarily expensive to synthesize. Thus,. . .

DETD In one preferred embodiment the **melting temperature** of the oligonucleotide probe that hybridize to the mutation locus is designed to have a lower **melting temperature** than the other oligonucleotide probe of the FRET oligonucleotide pair. Accordingly, the loss of fluorescence from the acceptor fluorophore (as the temperature of the sample is raised) will correspond to the **melting temperature** of the duplex formed at the locus containing the mutation/polymorphism. In one embodiment, both the donor oligonucleotide probe and the. . .

DETD In accordance with the present invention, **multiple loci** of a target nucleic acid sequence can be analyzed in a single vessel by designing sets of FRET oligonucleotide pairs. . .

DETD In accordance with one embodiment, the method of analyzing **multiple loci** uses a mixture of FRET oligonucleotide pairs that are labelled with different fluorescent resonance energy transfer pairs that have distinguishable. . .

DETD . . . sequences the fluorescent resonance energy transfer pairs are placed in fluorescent resonance energy transfer relationship. In one preferred embodiment the **melting temperature** of the second labeled oligonucleotide is higher than the **melting temperature** of the first and third labeled oligonucleotides..

DETD . . . of the third labeled oligonucleotide and the 3' end of the second labeled oligonucleotide. In accordance with this embodiment the **melting temperature** of the first labeled oligonucleotides is different from the **melting temperature** of the third labeled oligonucleotide. In one preferred embodiment, the donor fluorophore is fluorescein and the acceptor fluorophore is Cy5. . .

DETD . . . a method of analyzing a biological sample comprising a nucleic acid sequence for the presence of mutations or polymorphisms at **multiple loci** of the nucleic acid sequence is conducted by determining the **melting temperature** of a hybridization probe that is complementary to the locus containing the mutation or polymorphism. The method is conducted in. . . a second

donor oligonucleotide probe and second acceptor oligonucleotide probe, amplifying the selected segment of the DNA and determining the **melting temperature** of each set of donor and acceptor oligonucleotide probes. In accordance with this procedure, the pair of oligonucleotide PCR primers. . .

DETD To distinguish the melting point peaks of the two sets of probes, the probes are designed so the **melting temperature** of each set of probes is different from the **melting temperature** of the other set of probes. In preferred embodiments the multiple sets of FRET oligonucleotide pairs are each labeled with the same fluorescent resonance energy transfer pair and each FRET oligonucleotide pair has a distinct **melting temperature** range.

DETD . . . where a hybridization probe can be designed, using standard techniques known to those skilled in the art, to differ in **melting temperature** when hybridized to mutant vs wild type. The hybridization probes will typically be designed to detect a single base pair. . .

DETD . . . depend primarily on GC content and length. If a probe is designed to hybridize internally to the PCR product, the **melting temperature** of the probe also depends on GC content, length, and degree of complementarity to the target. Plotting fluorescence as a. . . GC/AT ratio, length, and sequence, and can be used to differentiate amplification products separated by less than 2.degree. C. in **melting temperature**. Thus continuous monitoring of fluorescence during the PCR reaction provides a system for detecting sequence alterations internal to the PCR. . .

DETD . . . and at least two sets of FRET oligonucleotide pairs as probes to simultaneously genotype the separate regions by analyzing the **melting temperature** of the sets of FRET oligonucleotide pairs. In this manner several different genes can be screened simultaneously in a single. . .

DETD The method of analyzing a biological sample for the presence of mutations or polymorphisms at **multiple loci** of nucleic acid sequences can be conducted in a single reaction vessel. In accordance with one embodiment, the method comprising. . .

DETD . . . probe in a resonance energy transfer relationship. In one embodiment, the first FRET oligonucleotide pair are labeled with the same **fluorescence resonance energy transfer** pair as the second FRET oligonucleotide pair, but the **melting temperature** of the first FRET oligonucleotide pair is different than the **melting temperature** of the second FRET oligonucleotide pair. In one embodiment the donor is fluorescein and the acceptor fluorophore is Cy5 or. . .

DETD . . . technique can be used to detect insertion and deletions in nucleic acid sequences by designing hybridization probes that have altered **melting temperature** when hybridized to a locus containing the mutation or polymorphism.

DETD . . . nucleic acid sequence wherein the hybridized set of first donor and first acceptor oligonucleotides are characterized as having a first **melting temperature**. The second donor oligonucleotide probe and second acceptor oligonucleotide probe are designed to hybridize to adjacent regions of a second. . . nucleic acid sequence wherein the hybridized set of second donor and second acceptor oligonucleotides are characterized as having a second **melting temperature**. Furthermore the oligonucleotide probes are designed so the first **melting temperature** of the set of first donor oligonucleotide probe and first acceptor oligonucleotide probe is different from the second **melting temperature** of the set of second donor oligonucleotide probe and second acceptor oligonucleotide probe.

DETD Alternatively, in one embodiment the additional sets of FRET oligonucleotide pairs are labeled with a **fluorescence resonance energy transfer** pair whose

acceptor fluorophore's emission does not overlap with the emission of the acceptor fluorophore of the first and second . . . have melting temperatures distinct from the first and second FRET oligonucleotide pairs as well as FRET oligonucleotide pairs labeled with **fluorescence resonance energy** **transfer** pairs whose acceptor fluorophores' emission does not overlap with the emission of the acceptor fluorophore of the first and second. . .

DETD In accordance with one embodiment of the present invention, **multiplex** PCR amplification and genotyping by fluorescent probe T.sub.m is used to simultaneously detect multiple variants in the hereditary hemochromatosis gene.. . .

DETD . . . dynamic monitoring of fluorescence as the temperature changes. In this manner mutations and polymorphisms can be detected by determining the **melting temperature** of added fluorescently labeled hybridization probes.

DETD **Multiplex** Analysis of the Hemochromatosis Gene

DETD **Multiplex** technology continues to advance both research and routine diagnostics. Sensitive methods of **multiplex** analysis combined with improved methods of DNA preparation increase the density of information obtained from small amounts of whole blood.. . .

DETD . . . DNA amplification to monitor hybridization is an extraordinarily powerful analytical technique that can be used to detect, simultaneously, mutations at **multiple loci**. Using the methods described herein and depending on the number of initial template copies present, screening for a specific known. . .

CLM What is claimed is:

. . . A method of analyzing a biological sample comprising a nucleic acid sequence for the presence of mutations or polymorphisms at **multiple loci** of the nucleic acid sequence, said method being conducted in a single reaction vessel and comprising the steps of (a). . .

. . . A kit for analyzing a biological sample comprising a nucleic acid sequence for the presence of mutations or polymorphisms at **multiple loci** of the nucleic acid sequence, said kit comprising: a. a mixture of a first donor oligonucleotide probe, a first acceptor. . . probe being designed to hybridize to adjacent regions of a second locus of the nucleic acid sequence so that the **melting temperature** of the set of first donor oligonucleotide probe and first acceptor oligonucleotide probe is different from the **melting temperature** of the set of second donor oligonucleotide probe and second acceptor oligonucleotide probe; b. a first pair of oligonucleotide primers. . .

. . . 10. A method of analyzing a biological sample comprising nucleic acid sequences for the presence of mutations or polymorphisms at **multiple loci** of the nucleic acid sequences, said method being conducted in a single reaction vessel and comprising the steps of (a). . .

=> d l3 7 kwic

L3 ANSWER 7 OF 10 USPATFULL

SUMM . . . defined sequence oligonucleotide, at a given stringency, hybridizes strongly (one manifestation of which is that the hybrids have a high **melting temperature**) to a target sequence with which it is complementary along its entire length (a perfect hybrid or perfect match), but. . .

DETD . . . form stronger hybrids (i.e., duplexes) than those formed by

unmodified oligonucleotides. Hybridization strength is generally assessed by determination of the **melting temperature** (T.sub.m) of a hybrid duplex. This is accomplished by exposing a duplex in solution to gradually increasing temperature and monitoring. . .

DETD . . . appropriate excitation wavelengths. This method has the advantage that released label does not have to be separated from intact probe. **Multiplex** approaches utilize multiple probes, each of which is complementary to a different target sequence and carries a distinguishable label, allowing. . .

DETD . . . some of these assays, fluorescence and/or changes in properties of a fluorescent label are used to monitor hybridization. For example, **fluorescence resonance energy transfer** (FRET) has been used as an indicator of oligonucleotide hybridization. In one embodiment of this technique, two probes are used,. . .

=> d l3 8 kwic

L3 ANSWER 8 OF 10 USPATFULL

SUMM . . . the two labels are very close to each other. When the sample is stimulated by light of an appropriate frequency, **fluorescence resonance energy transfer** ("FRET") from one label to the other occurs. This energy transfer produces a measurable change in spectral response, indirectly signaling. . .

SUMM The measurable characteristic may be a characteristic light signal that results from stimulating at least one member of a **fluorescence resonance energy transfer** (FRET) pair. It may be a color change that results from the action of an enzyme/suppressor pair or an enzyme/cofactor. . .

DETD . . . probe 1 is in the open state (FIG. 2), label moiety 6 is sufficiently separated from label moiety 7 that **fluorescence resonance energy transfer** between them is substantially, if not completely, precluded. Label moiety 6 is therefore unable to quench effectively the fluorescence from. . .

DETD . . . is governed by two criteria related to the thermodynamics of probes according to the invention. First, we prefer that the **melting temperature** of the arm stem, under assay conditions, be higher than the detection temperature of the assay. We prefer stems with. . .

DETD . . . formation of the target complement sequence-target sequence hybrid so that target-mediated opening of the probe is thermodynamically favored. Thus, the **melting temperature** of the target complement sequence-target sequence hybrid is higher than the **melting temperature** of the stem. Therefore, arm sequences should be shorter than the target complement sequence. For bimolecular embodiments, as already stated,. . .

DETD Therefore, the **melting temperature** of the arm stem must be above the assay temperature, so that the probe does not open before the target complement sequence hybridizes to a target, and yet sufficiently below the **melting temperature** of the hybrid, complete or nicked, of the target complement sequence with the target sequence to assure proper probe functioning and, thereby, generation of a detectable signal. We prefer that the **melting temperature** of the arm stem be at least 5.degree. C., more preferably at least 10.degree. C., above the assay temperature and at least about 20.degree. C. below the **melting temperature** of the hybrid of the target complement sequence with the target sequence.

DETD . . . conditions but not under another set of assay conditions. The

length of the arms and their guanosine-cytidine content affect the **melting temperature** of a stem duplex. For a desired **melting temperature**, under particular assay conditions, a length and a guanosine-cytidine content of the arms can easily be calculated by those skilled in the art. The **melting temperature** of the duplex stem of a probe can be empirically determined for given assay conditions using the methods described below.

. .

DETD Our preferred labels are chosen such that **fluorescence resonance energy transfer** is the mode of interaction between the two labels. In such cases, the measurable physical characteristics of the labels could. . .

DETD . . . used in the art. The alkyl spacers give the label moieties enough flexibility to interact with each other for efficient **fluorescence resonance energy transfer**, and consequently, efficient quenching. The chemical constituents of suitable spacers will be appreciated by persons skilled in the art. The. . .

DETD . . . described below. We have prepared the two universal stem oligonucleotides by solid-state synthesis. However, natural sequences of appropriate length and **melting temperature** may also be adapted for use as stems.

DETD . . . complement sequence and form the appropriate linkable terminus, described above. A kit may include multiple universal stems varying by the **melting temperature** and/or length of the final probe stem to be formed. A kit could have one common stem oligonucleotide and multiple. . .

DETD . . . to an expected amplification product, is included in a polymerase chain reaction mixture. For this embodiment the probe has a **melting temperature** such that the probe remains closed under the reaction conditions at the annealing temperature of the polymerase chain reaction. The. . .

DETD Probes of this invention exhibit a characteristic **melting temperature**, Tm, the temperature at which two hybridized nucleic acid strands separate due to thermal energy. The **melting temperature** of Probe A was determined by monitoring the level of its fluorescent signal as temperature was increased from 10.degree. C.. . . these assay conditions. The Tm of a probe is indicated by the inflection point of its thermal denaturation curve. The **melting temperature**, Tm, of Probe A was 27.degree. C.

DETD . . . duplex and at high temperatures the helical order of the stem melted, and the probe assumed a random-coil configuration. The **melting temperature** of robes according to this invention depend upon the length and the guanosine-cytosine content of the arm sequences and the. . . target complement sequences but different arms and different test assay conditions. Divalent cations have a particularly powerful influence upon the **melting temperature**. For example, the **melting temperature** of Probe A (FIG. 3), was 27.degree. C. in the absence of magnesium ions, but was 56.degree. C. in the. . .

DETD . . . an incubation step used in this reaction. An additional temperature in each cycle, which is 5-12.degree. C. lower than the **melting temperature** of the stem of the probe, can be included as the detection temperature. In each cycle, the level of fluorescence. . .

CLM What is claimed is:

10. A **multiplex** assay for at least two different nucleic acid target sequences comprising the steps of adding to a sample suspected to. . .

=> d 13 9 kwic

L3 ANSWER 9 OF 10 PATFULL

SUMM Clegg, R., (1992) "**Fluorescence resonance energy transfer** and nucleic acids", Meth. Enzymol. 211:353-388.

SUMM . . . P., Bloch, W., Brinson, E., Chang, C., Eggerding, F., Fung, S., Iovannisci, D., Woo, S. and Winn-Deen, E. (1994) "High-density **multiplex** detection of nucleic acid sequences: oligonucleotide ligation assay and sequence-coded separation" Nucl. Acids Res. 22:4527-34.

DETD . . . nt. The assembly and bridging oligonucleotides comprising the assembled gene are selected according to the predicted annealing properties, i.e. thermal **melting temperature**, T.sub.m. The duplex regions resulting from annealing of the oligonucleotides must be stable enough to endure the washing step, and. . .

DETD . . . PCR products are labeled with different fluorescent dyes, the multiple PCR products can be spectrally discriminated, thereby detected and quantitated. **Multiplex** PCR on solid-support is also a convenient, efficient way to handle templates for PCR on solid-support, giving rise to less. . .

DETD . . . portion of the immobilized ligation product. The probe includes a fluorescent reporter dye and quencher arranged to interact through a **fluorescence resonance energy transfer** (FRET) effect (Clegg, R., 1992). The quencher can interact with the reporter to alter its light emission, usually resulting in. . .